

Amendments to the Specification

Please insert the sequence listing being filed concurrently herewith into the specification.

Please replace the paragraph beginning on page 24, line 25, and ending on page 25, line 10, with the following new paragraph:

The cloning steps required for the construction of this *ori*SELECT strain are described in more detail below:

Splicing PCR to produce the RNAII-lacI fusion

1. Amplify part of the 5' end of RNAII from pUC18 by PCR using the following primers (product = 176 bp), as shown in Figure 5.

5RNAII:GAATGCATCAAAGGATCTTCTTGAGA (26 nt) (SEQ ID NO:1)

3RNAII:ACATTCACCACCGTATTTGGTATCTGCGCTCTGCTGAAGCCA (42 nt)
(SEQ ID NO:2).

2. Amplify part of the 5' end of *lacI* from DH1 gDNA by PCR using the following primers (product = 597 bp), as shown in Figure 6

5lacI: GATACCAAATACGGTGGTGAATGTGAAACCAGTAACGTTATA
(42 nt) (SEQ ID NO:3)

3lacI: ACAGAACTTAATGGGCCCGCTAACA (25 nt) (SEQ ID NO:4).

3. Combine the two PCR products in a single PCR and amplify using 5RNAII and 3lacI primers to generate the 749 bp RNAII-*lacI* gene fusion PCR product (the region between 5RNAII and 3lacI primers below), as shown in Figure 7.
4. Cut the spliced PCR product with *NsiI* and *ApaI* to generate the fragment for cloning into the lactose operon in place of the *lacI* promoter and 5' end.

Please replace the paragraph spanning lines 12 to 25 of page 25 with the following new paragraph:

Cloning part of the lactose operon and inserting RNAII-*lacI*

1. Amplify the *lac* operon from DH1 gDNA with the following primers.
5LO: CTCTTGCGCCGGGTCGACATACCCC (25 nt) (SEQ ID NO:5)
3LO: TAAGTCGACCACGGGTGCCGTTTT (25 nt) (SEQ ID NO:6)
Primer 5LO incorporates a natural *SalI* site (underlined), whilst 3LO introduces one with a single nucleotide change (bold). Total PCR product size = 5803 bp.
2. Cut the PCR product with *SalI* and clone into pUC18 cut with the same.
3. Cut this plasmid with *NsiI* and *ApaI* and clone in the fragment from step 4, replacing the promoter and 5' end of *lacI* with the RNAII-*lacI* gene fusion.
4. Cut insert with *SalI* and ligate into pKO3recA cut with same.
5. Integrate into the chromosome of DH1*lacdapD*, DH1*lacP2dapD* and DH1*lackan* to produce *oriSELECT* strains.
6. Test plasmid selection and maintenance using plasmids with pMB1 origins of replication.